

On some actinocephalid gregarines (Apicomplexa : Eugregarinida) of Odonate insects from West Bengal, India

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Abstract. The morphology and life history of three species of cephaline gregarines (Apicomplexa : Eugregarinida) have been described in this paper. These are *Mukundaella undulatus* gen. nov. sp. nov., *Odonaticola elliptica* sp. nov. and *O. nonacontha* (Devdhar and Deshpande) comb. nov. from the midguts of *Enallagma* sp., *Crocothemis s. servilia* (Drury) and *Urothemis s. signata* (Rambur) respectively. A comparative study of the various genera having biconical spore with polar and meridional spines has also been made with the new genus *Mukundaella* gen. nov. to establish its distinctiveness.

Keywords. *Mukundaella undulatus*; *Odonaticola elliptica*; *O. nonacontha*; Apicomplexa; Eugregarinida; Odonate.

1. Introduction

The fauna of cephaline gregarines from insects especially of Coleoptera and Orthoptera have been extensively studied throughout the world. However, the cephaline gregarines from Odonata have not been adequately investigated. The available limited information has been contributed by K  lliker (1848), L  ger (1892) Ellis (1914), Kamm (1922), Foerster (1938), Hoside (1953), Obata (1953), Stein (1960), Desportes (1963) and Geus (1969). In India, the study of cephaline gregarines from odonates has been initiated by Sarkar and Chakravarty (1969), and continued by Devdhar and Deshpande (1971), Sarkar (1978), Nazeer Ahamed and Narasimhamurti (1979) and Sarkar and Haldar (1980, 1981a, 1981b, 1981c, 1981d and 1981e).

This paper records three species of cephaline gregarines from odonates of West Bengal, India, of which two have been considered as new to science while the third one has been assigned to a new taxonomic status.

2. Material and methods

The insects were collected from several agricultural fields as well as from nearby places of lakes and brought alive to the laboratory. The alimentary canals were

dissected out on clean glass slides with 0.5% saline under a dissecting binocular and were examined for the parasites. The smears made on clean glass slides from the infected midguts, were fixed in Schaudinn's and Bouin's fixatives. The 5.0 μm thick serial sections of the Bouin's fixed heavily infected midguts were made for the intracellular stages of the parasites. The slides containing smears and sections were subsequently stained with iron alum haematoxylin method. The isolated gametocysts were placed in the moist chamber to study their further development. The fresh spores were examined with Lugol's iodine. The abbreviations used here are D = deutomerite, E = epimerite, L = length, N = nucleus, Nc = neck, P = protomerite, T = total and W = width. The ratios used are the ratio of the length of protomerite to the total length (LP:TL) and the ratio of the width of protomerite to the width of deutomerite (WP:WD). All figures have been drawn with the aid of camera lucida.

3. Observations

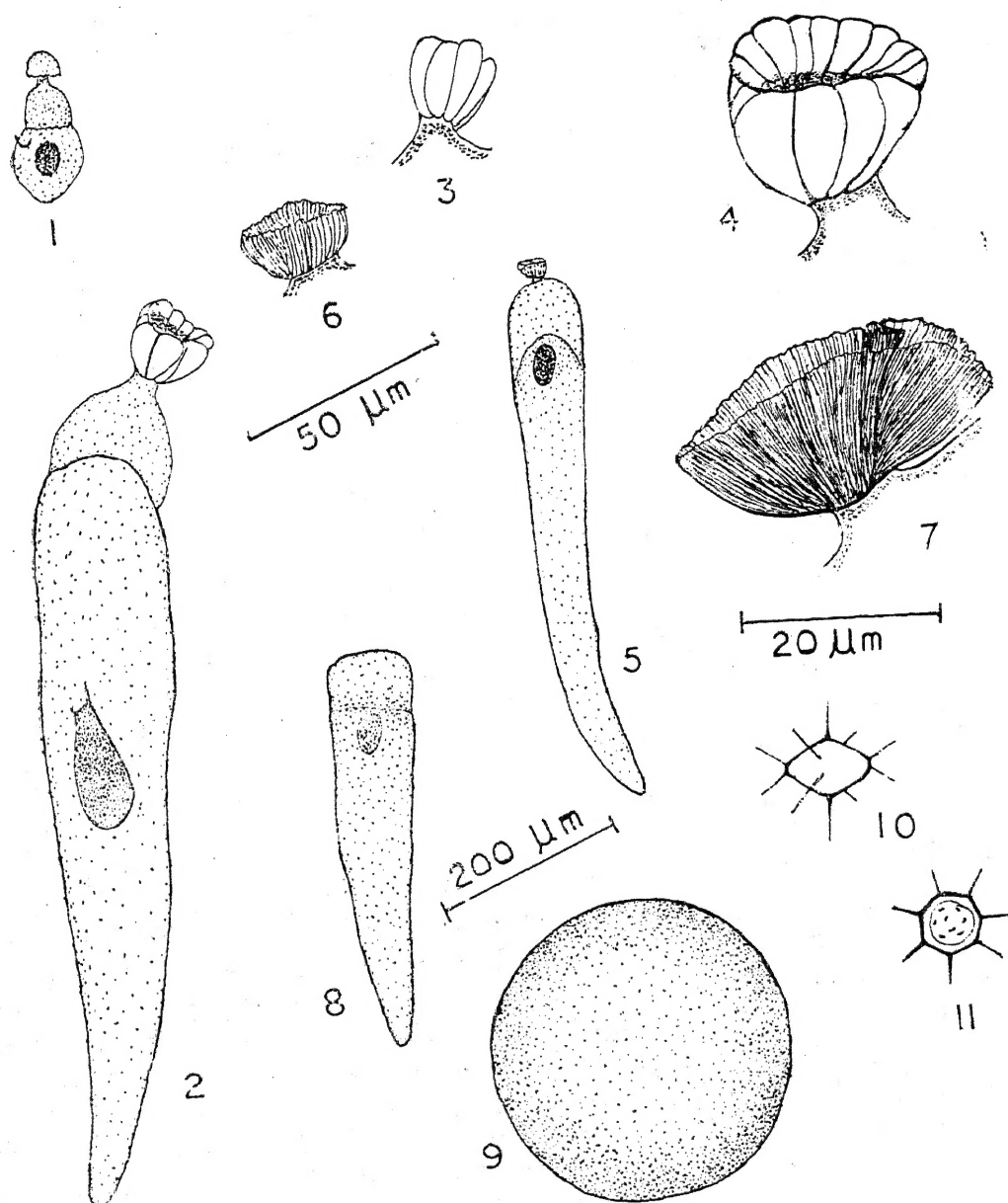
3.1. *Mukundaella undulatus* gen. and sp. nov.

Of the 65 insects examined from Chinsurah and Naihati of West Bengal, 13 were found to be infected by the gregarine showing 20.0% incidence.

3.1a. *Trophozoite*. The earliest stage obtained from the midgut content of *Enallagma* sp. is a three-segmented trophozoite (figure 1) having a small hemispherical epimerite with a short neck, a dome-shaped protomerite and an almost fusiform deutomerite with an ovoidal nucleus at the centre. The earliest forms measure 45.4 μm $\times$ 15.2 μm on the average. The trophozoites, when fully grown, become elongated, fusiform or cylindroconical and measure 348.6 μm $\times$ 50.5 μm on the average (figures 2 and 5). The epimerite at its early stage of the fully grown trophozoites is a cup having a few vertical folds on its wall and becomes connected with the conical protomerite through a short neck (figures 3 and 6). When fully differentiated, the epimerite becomes broader and wider having numerous closely-set vertical undulations on its wall (figures 4 and 7). The fully grown epimerites measure 17.0 μm $\times$ 28.4 μm on the average. The protomerite is conical or rectangular while the deutomerite is fusiform or cylindroconical having a central or anteriorly placed nucleus. The septum between the protomerite and the deutomerite is distinct.

3.1b. *Sporadin*. The sporadins are solitary having an almost rectangular protomerite and a cylindroconical deutomerite (figure 8). The nucleus is recognized in the anterior part of the deutomerite. It is ellipsoidal or ovoidal with a distinct nuclear membrane but no conspicuous endosomes are discernible. The nucleus measures 40.0 μm $\times$ 19.0 μm on the average.

3.1c. *Gametocyst and spore*. The gametocysts, collected from the midgut as well as hindgut of the hosts, are milky-white spherical bodies having a thin but distinct cyst wall, 300.0 μm in diameter (figure 9). After seven days of development in the moist chamber, the spores are liberated by simple rupture of the gametocyst wall. The spores are diamond-shaped (figure 10), hexagonal in polar view (figure 11), having two polar and six meridional spines and measured 8.5 μm $\times$ 5.0 μm .



Figures 1-11. The various stages of *Mukundaella undulatus* gen. nov. sp. nov. 1. An early trophozoite. 2. A fully grown trophozoite with a few undulations in the epimerite. 3 and 4. Epimerites at early stages of differentiation of mature trophozoite. 5. A fully grown trophozoite with numerous undulations in the epimerite. 6 and 7. Fully differentiated epimerite of mature trophozoite. 8. A sporadin. 9. A spherical gametocyst. 10 and 11. Spores at meridional and polar view respectively.

3.1d. *Measurement* (in μm). The detail of measurements of the trophozoites and sporadins is given in table 1.

3.1e. *Discussion*. The present gregarine, in having biconical spore with one row of polar and one row of meridional spines, clearly resembles the genera *Acanthospora* Léger, *Ancyrophora* Léger and *Ramicephalus* Obata of the sub-family Acanthosporinae Léger emend Grasse. These genera are distinguished from each

Table 1. The details of the measurements (in microns) of *Mukundaella undulatus* gen. nov. sp. nov.

Serial No.	TL	LE	LNc	LP	LD	LN	WE	WP	WD	WN	LP:TL	WP:WD
1.	46.0	6.0	4.0	12.0	24.0	..	8.0	10.0	14.0	..	1:3.8	1:1.4
2.	57.0	5.0	4.0	16.0	32.0	12.0	8.0	12.0	16.0	6.0	1:3.6	1:1.3
3.	38.0	7.2	2.8	12.0	16.0	6.8	8.0	12.0	16.0	6.0	1:3.2	1:1.3
4.	38.0	6.0	2.0	10.0	20.0	8.0	8.0	10.0	14.0	6.0	1:3.8	1:1.4
5.	48.0	8.0	4.0	16.0	24.0	6.0	8.0	12.0	16.0	4.8	1:3.0	1:1.3
6.	225.4	16.7	8.3	25.0	175.4	25.0	20.0	33.4	33.4	10.0	1:9.0	1:1.0
7.	284.0	16.7	8.3	41.7	217.3	33.4	21.7	46.8	58.4	16.7	1:6.8	1:1.2
8.	484.3	14.2	6.7	46.8	416.6	33.4	33.4	53.4	43.4	21.7	1:10.3	1:0.8
9.	400.0	20.0	6.7	43.4	329.0	...	38.4	50.0	66.8	...	1:9.2	1:1.3
10.	384.0	...	...	41.7	342.3	33.4	...	83.5	75.0	16.7	1:9.2	1:0.9
11.	350.7	...	...	50.1	300.6	...	...	133.6	142.0	...	1:7.0	1:1.1
12.	267.2	...	...	83.5	183.7	33.4	...	50.0	50.0	25.0	1:3.2	1:1.0
13.	467.6	...	...	50.0	417.6	50.0	...	50.0	83.5	16.7	1:9.3	1:1.7
14.	434.2	...	...	50.0	384.2	33.4	...	117.0	117.0	20.0	1:8.6	1:1.0
15.	451.0	...	...	83.5	367.5	50.0	...	50.0	66.8	16.7	1:5.4	1:1.2

LP : TL 1 : 3.0-10.3 (6.36) ; WP : WD = 1 : 0.8-1.7 (1.2).

other in the structure of their epimerite, viz., simple knob in *Acanthospora*, digitiform ball or disc in *Ancyrophora* and dendroidal disc in *Ramicephalus*. However, the epimerite of the gregarine under report is markedly distinct from the above genera in having a broad and wide cup with numerous vertical undulations and sets the species apart from all the known genera. A new genus *Mukundaella* gen. nov. is, therefore, proposed after the late Prof. Mukunda M. Chakravarty, an illustrious protozoologist of India, to accommodate it and has been described as *Mukundaella undulatus* gen. nov. sp. nov. A comparison of the various genera having biconical spore with polar and meridional spines has been made with the genus *Mukundaella* gen. nov. in table 2 to establish its distinctiveness.

3.1f. *Diagnosis.* *Mukundaella undulatus* gen. nov. sp. nov. Trophozoites elongate, cylindroconical; epimerite a broad wide cup with numerous vertical undulations on its wall, placed on a short neck; sporadins large, cylindroconical, solitary; gametocysts spherical, sporulation by simple rupture; spores diamond-shaped, hexagonal in polar view, with polar and meridional spines.

Seat of infection: Midgut.

Host: *Enallagma* sp.

Locality: Chinsurah and Naihati of West Bengal, India.

3.2. *Odonatticola elliptica* sp. nov.

The gregarine has been obtained from the midgut of *Crocothemis s. servilia* (Drury). Of the 50 insects examined, 14 have been found to be infected showing 28.0% incidence.

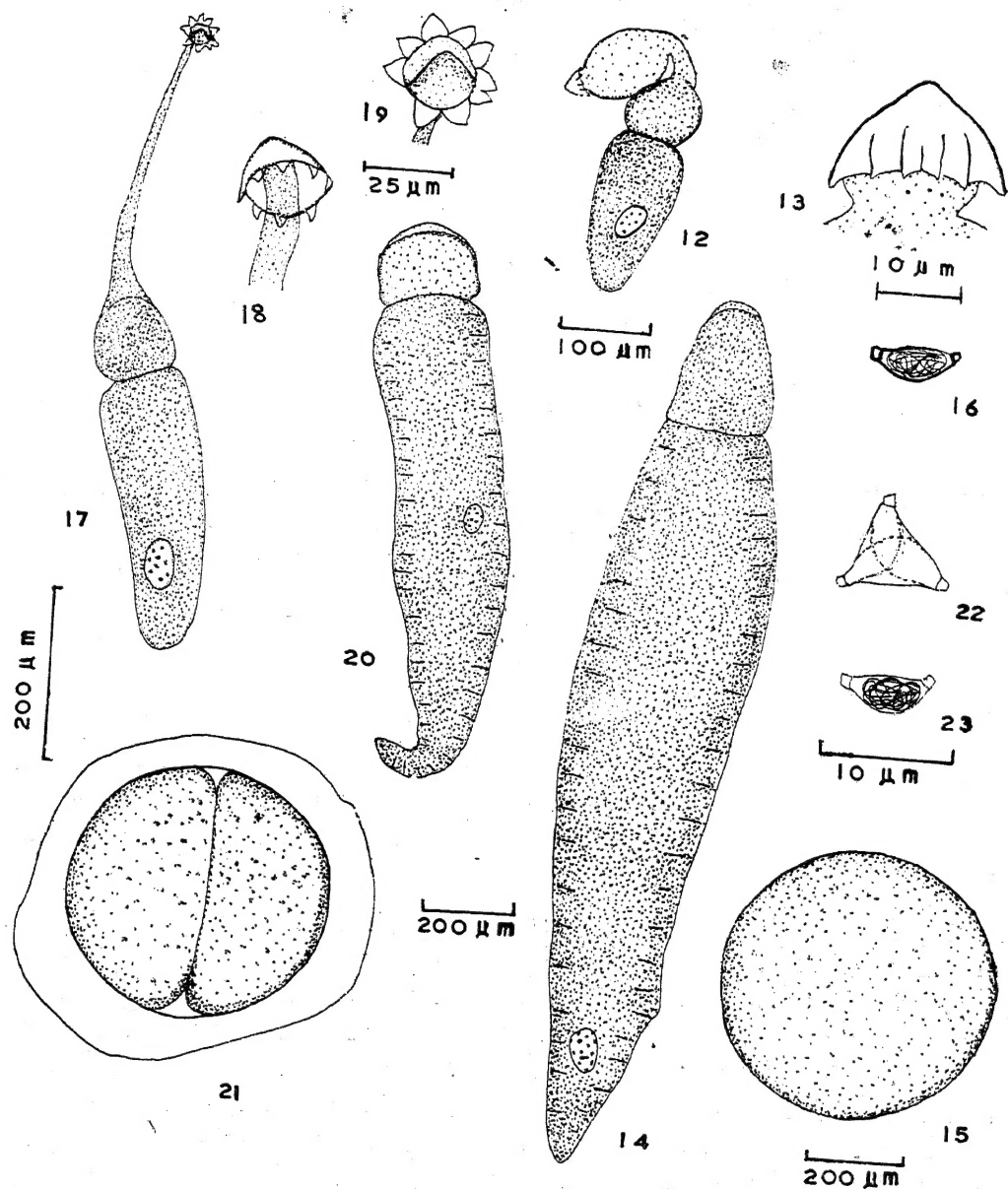
Table 2. A comparative study of the different genera having biconical spores with polar and meridional spines.

Genera	Characters	
	Epimerite	Spore
<i>Acanthospora</i> Léger 1892	Non-appendiculate, simple knob ;	Biconical, one row of polar and meridional spines ;
<i>Ancyrophora</i> Léger 1892	Globular, five to twelve backwardly directed digitiform processes ;	Biconical, one row of polar and meridional spines ;
<i>Cometoides</i> Labbé 1899	Globular, six to eight long filaments directed upwards ;	Cylindrobiconical, one row of polar and two rows of meridional spines ;
<i>Ramicephalus</i> Obata 1953	Discoid, many upwardly directed dendroidal processes ;	Biconical, one row of polar and meridional spines ;
<i>Mukundaella</i> gen. nov.	Broad, wide cup with numerous closely-set vertical undulations ;	Diamond-shaped, hexagonal in polar view, two polar and six meridional spines.

3.2a. *Trophozoite*. The trophozoites are three-segmented gregarine having a small, conical umbrella-like epimerite with many small, curved spines along its margin, an ovoid protomerite and a fusiform deutomerite with an ellipsoidal nucleus at its centre (figures 12 and 13). The epimerite is bridged with the protomerite through a broad neck. The anterior part of the neck is elliptic in outline (figure 12) and measures $73.8 \mu\text{m} \times 51.3 \mu\text{m}$ on the average. The septum between the protomerite and neck is not so distinct as it is between the protomerite and deutomerite. On the average the trophozoites measure $309.4 \mu\text{m} \times 62.5 \mu\text{m}$.

3.2b. *Sporadin*. The sporadins are large, solitary forms and measure $1512.0 \mu\text{m} \times 294.6 \mu\text{m}$ on the average (figure 14). The protomerite is conical while the deutomerite is almost fusiform embodying an ovoidal nucleus in it. The deutomerite exhibits numerous horizontal lines in its cytoplasm which are supposed to be myoneme fibrils. The nucleus is large and ovoid with distinct nuclear membrane and many small round or ovoidal endosomes. The nucleus measures $82.3 \mu\text{m} \times 50.0 \mu\text{m}$ on the average.

3.2c. *Gametocyst and spore*. The gametocysts are milky-white, spherical bodies with a thin cyst wall and measure $510.0 \mu\text{m}$ in diameter on the average (figure 15). The spores are liberated by a simple rupture of the cyst wall. Each spore is boat-shaped having filiform sporozoites inside and measures $12.0 \mu\text{m} \times 5.0 \mu\text{m}$ (figure 16).



Figures 12-23. The various stages of *Odonaticola elliptica* sp. nov. 12. A fully grown trophozoite. 13. A conical epimerite with many curved spines. 14. A fully grown sporadin. 15. A spherical gametocyst. 16. A boat-shaped spore with filiform sporozoites. 17-23. The various stages of *O. nonacontha* (Devdhar and Deshpande) comb. nov. 17. A fully grown trophozoite. 18. A conical epimerite—side view. 19. Epimerite with nine petaloid spines—viewed from above downward. 20. A fully grown sporadin. 21. An early gametocyst with ectocyst. 22. Spores in cluster of three. 23. A boat-shaped spore with filiform sporozoites.

3.2d. *Measurement* (in μm). The measurements of various body parts (trophozoites and sporadins) with the mean within parenthesis are given below: .

TL = 233.5 - 1700.3 (910.7); LNC = 75.0 - 145.9 (107.2);
 LE = 12.5 - 18.8 (15.8); WE = 16.7 - 22.9 (20.0);
 LP = 45.9 - 233.4 (126.9); WP = 41.7 - 250.0 (122.9);

LD = 104.2 – 1483.6 (724.5); WD = 50.0 – 383.4 (178.5);
 LN = 33.4 – 100.0 (60.3); WN = 28.8 – 58.3 (37.9);
 LP: TL = 1:7.2 and WI:WD = 1:1.4.

3.2e. *Affinity*. The features such as the conical umbrella-like epimerite with small, curved spines; long neck, boat-shaped spores and the odonate host of this gregarine resemble the diagnostic features of the genus *Odonaticola* Sarkar and Haldar (1981a). However, the elliptic anterior part of its neck and the measurement of various body parts warrant that it be considered a new species for which the name *Odonaticola elliptica* sp. nov. is proposed.

3.3. *Odonaticola nonacontha* (Devdhar and Deshpande) comb. nov.

The gregarine has been obtained from the midgut of *Urothemis s. signata* (Rambur) from Hooghly, West Bengal. Out of 121 insects examined during 1978-1979, 30 insects have been found to be infected showing 24.8% incidence.

3.3a. *Trophozoite*. The trophozoites consist of a conical epimerite with a very long neck, a dome-shaped protomerite and a cylindroconical deutomerite having an ellipsoidal nucleus in it (figure 17). The conical epimerite is provided with nine petaloid spines along its margin (figures 18 and 19).

3.3b. *Sporadin*. The sporadins are solitary having a dome-shaped protomerite and a cylindroconical deutomerite (figure 20). The deutomerite exhibits numerous horizontal myonemes in its cytoplasm and embodies an ellipsoidal nucleus with many endosomes in it.

3.3c. *Gametocyst and spore*. The gametocysts are milky-white, spherical and 300.0 μm in diameter on the average (figure 21). After five days in the moist chamber, the gametocysts burst by simple rupture of the cyst wall and the spores are released mostly in clusters (figure 22) and a few singly. Each spore is boat-shaped and measures 8.5 μm $\times$ 4.5 μm (figure 23).

3.3d. *Measurement* (in μm). The measurement of various body parts (trophozoites and sporadins) with the mean within parenthesis is given below:

TL = 283.4 – 1633.7 (973.6); LNC = 125.0 – 216.8 (170.9);
 LE = 22.9 – 25.0 (24.2); WE = 27.0 – 29.2 (28.3);
 LP = 66.7 – 216.7 (135.0); WP = 66.7 – 200.0 (142.6);
 LD = 216.7 – 1416.9 (743.9); WD = 100.0 – 266.7 (173.2);
 LN = 58.3 – 100.0 (69.2); WN = 29.2 – 66.7 (43.2);
 LP: TL = 1:7.2 and WP:WD = 1:1.2.

3.3e. *Discussion*. In having nine petaloid spines along the margin of the conical epimerite, very long neck, numerous horizontal myonemes in the deutomerite, spherical gametocyst and the odonate host, the present gregarine resembles *Menospora nonacontha* Devdhar and Deshpande (1971). Further, the measurements of various body parts of the two species are also very closely related. Therefore, these two species appeared to be the same. Although the gregarine reported by Devdhar and Deshpande has been placed under the genus *Menospora* Léger (1892),

it does not possess any of the diagnostic features of the genus, viz., cup-like epimerite with recurved digitiform processes or hooks and crescentic spores. (Devdhar and Deshpandes' claim that the epimerite of *M. nonacontha* is a cup with nine recurved hooks, is possibly the result of an artifact and wrong observations as is evident when their illustration has been compared with the present one.) In fact, the two species, which I claim, are the same and more akin to the genus *Odonaticola* Sarkar and Haldar (1981a) in having boat-shaped spores and other features of the gregarine mentioned earlier. The present species has, therefore, been described as *Odonaticola nonacontha* (Devdhar and Deshpande) comb. nov.

4. Materials

Holotypes of the gregarines reported in this paper, prepared from the midgut content of their respective hosts, are deposited at present in the Department of Zoology, RBC College, Naihati and soon, will be sent to the National Collection of the Zoological Survey of India, Calcutta.

5. Prevalence

During the period of investigation, the rate of infection has been found to be very high at the end of the rainy season. The number of gregarine per infected host has also been found maximum during the same period. Otherwise, the infection is scanty throughout the year.

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In this paper, Classification of Protozoa given by Levine *et al* published in *J. Protozool.* 27 (1) 1980 has been followed.

